PRECLINICAL DATA SUPPORTING THE INITIATION OF THE EDIT-301 PHASE I/II RUBY CLINICAL TRIAL FOR THE POTENTIAL TREATMENT OF SICKLE CELL DISEASE

Edouard De Dreuzy[1], Jack Heath[1], Patricia Sousa[1], Tusneem Janoudi[1], Dawn Ciulla[1], Georgia Giannoukos[1], Pavlina Wolf[1], Harry An[1], Meng Wu[1], Tongyao Wang[1], Eugenio Marco[1], Scott Hansen[2], David K. Wood[2], Sandra Teixeira[1], Tamara T. Monesmith[1], Kate Zhang[1], Kai-Hsin Chang[1]

[1]Editas Medicine, Inc., Cambridge, MA
[2]University of Minnesota, Minneapolis, MN

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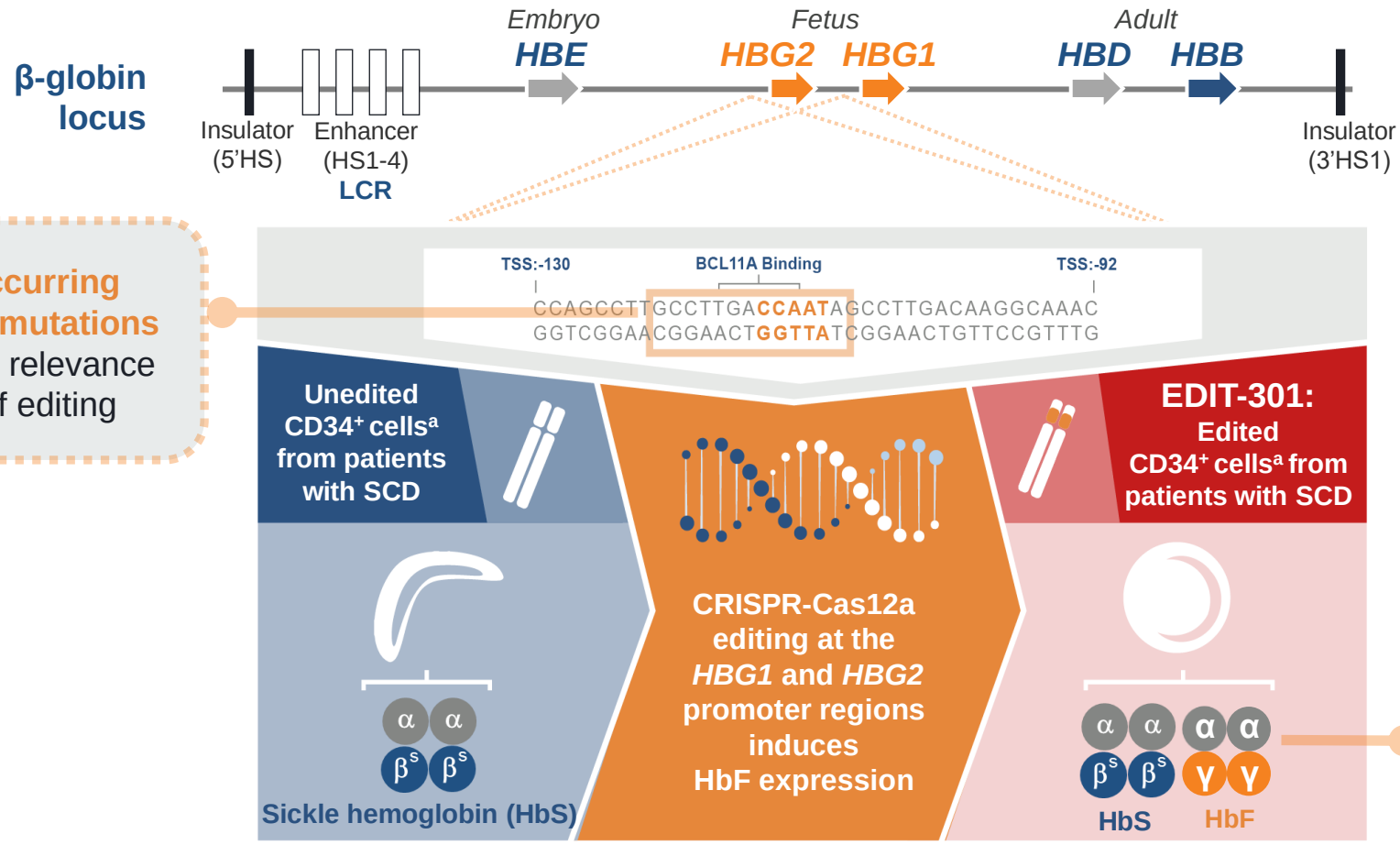
Disclosures

Edouard De Dreuzy is a full-time employee and shareholder in Editas Medicine

Naturally Occurring Mutations Support Clinical Relevance and Safety of Editing at the *HBG1/2* Promoter Region



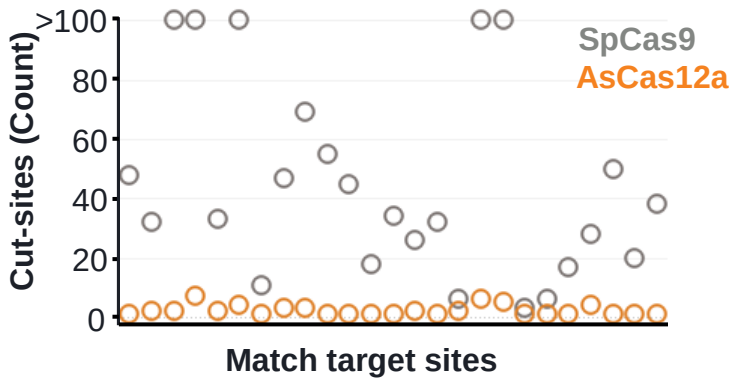
Naturally occurring HbF-inducing mutations support clinical relevance and safety of editing

HbF production reduces RBC sickling by inhibiting formation of HbS fibers at low oxygen levels

[a]CD34+ hematopoietic stem and progenitor cell
HS: hypersensitive site; LCR: locus control region; TSS: transcriptional start site
Adapted from Higgs, Engel and Stamatoyannopoulos. *Lancet* 2012

EDIT-301 Editing using highly specific and potent CRISPR-Cas12a enzyme

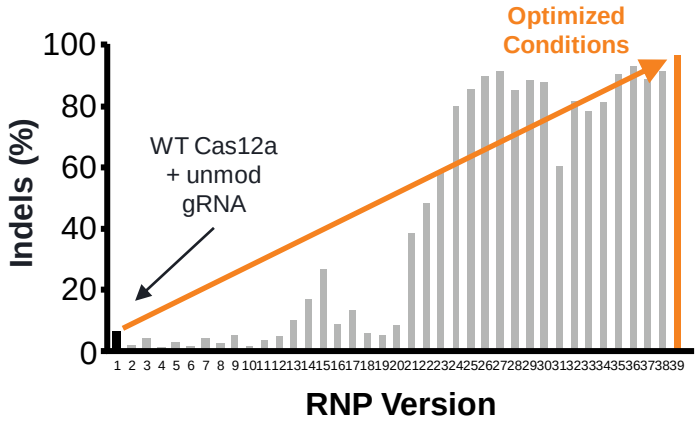
Editing Specificity



AsCas12a is Highly Specific primarily due to its DNA target engagement mechanism that is distinct from SpCas9

Gotta et al. Cold Spring Harbor 2019

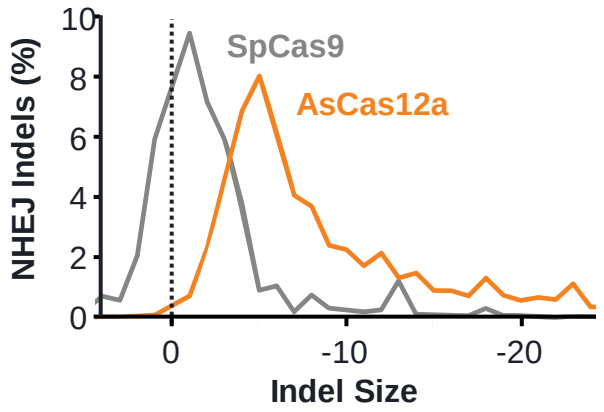
Editing Efficiency



Editas engineered AsCas12a RNP demonstrates **high editing efficiency** in CD34+ cells

De Dreuzy et al, ASH 2019

Indel Profile



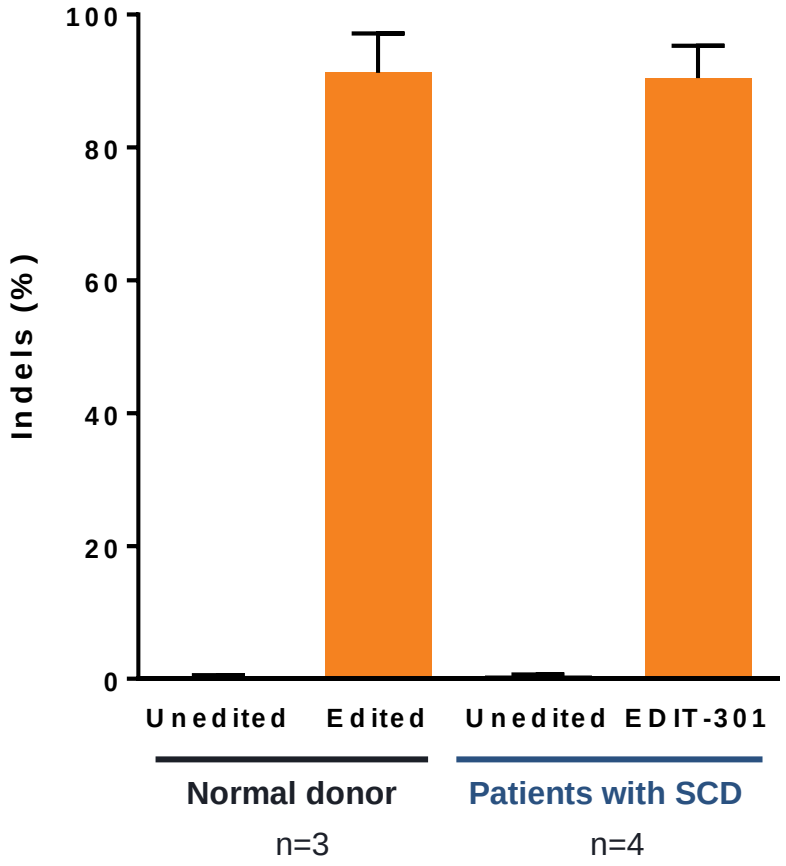
AsCas12a staggered cut generate larger deletions than SpCas9, **leading to higher HbF induction** at the HBG locus

De Dreuzy et al, ASH 2019
De Dreuzy et al, ASGCT 2018

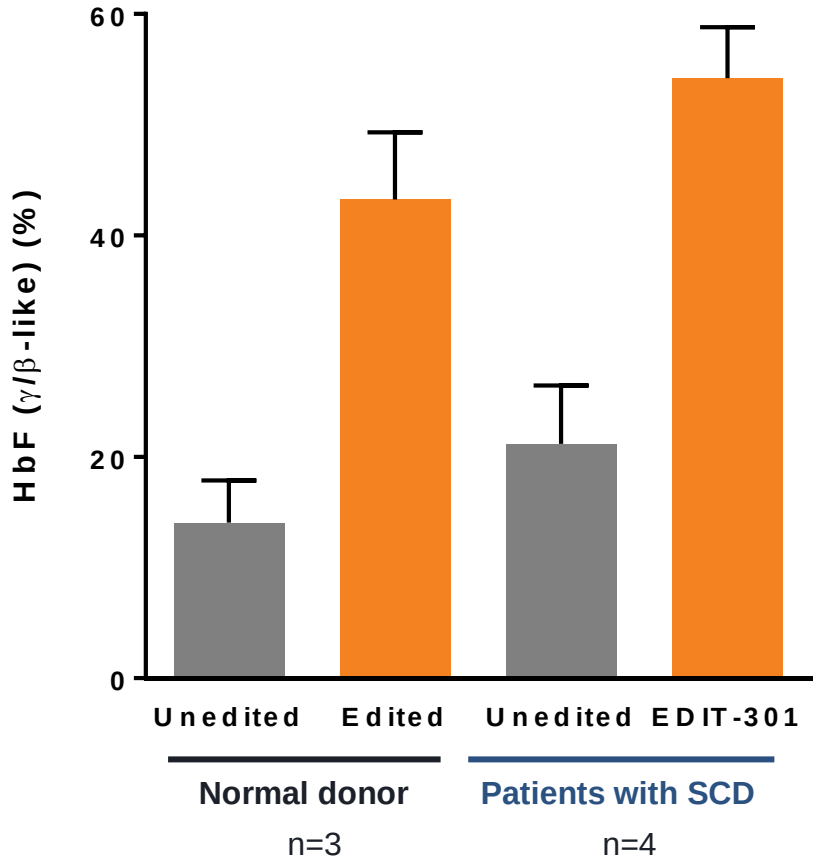
Match target Site are 20mer protospacer sequence within the human genome with compatible PAM's for different nucleases (20-Ns)

High level of editing and robust HbF induction in edited CD34+ cells from normal donors and patients with Sickle Cell Disease (SCD)

Efficient editing



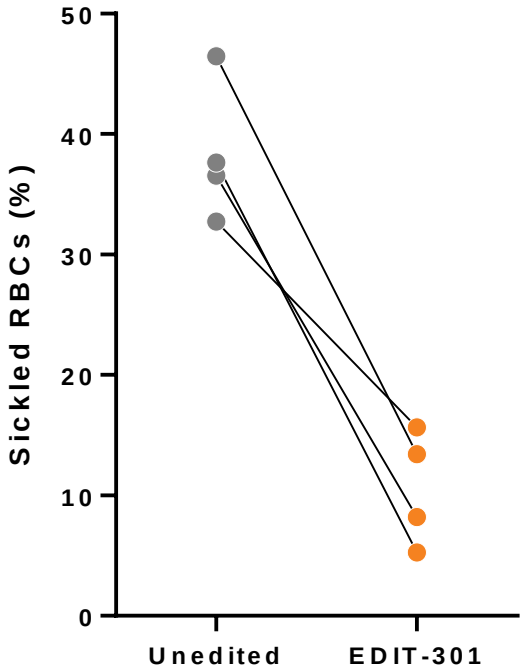
Robust *ex vivo* HbF expression



Unedited cells did not undergo electroporation

EDIT-301-derived RBCs have reduced sickling and improved rheological properties versus unedited SCD-derived RBCs

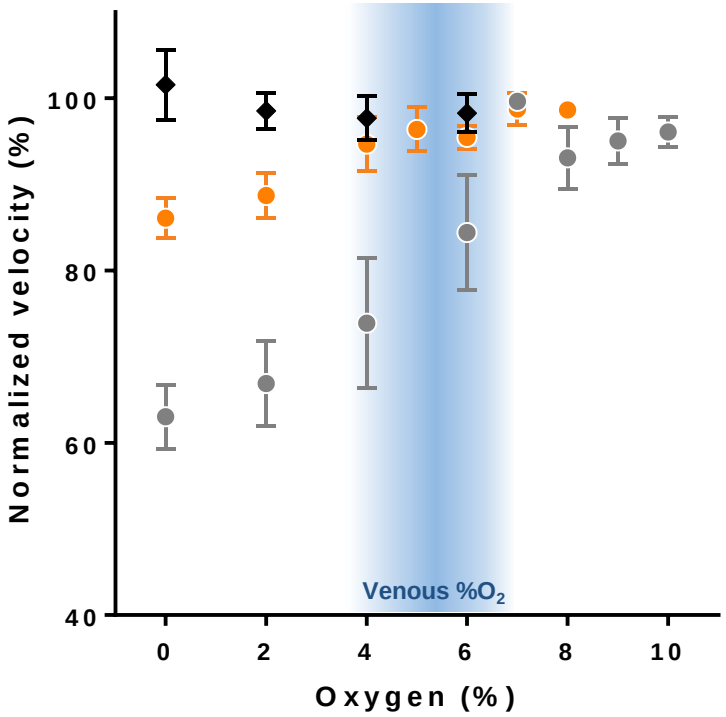
Reduced sickling



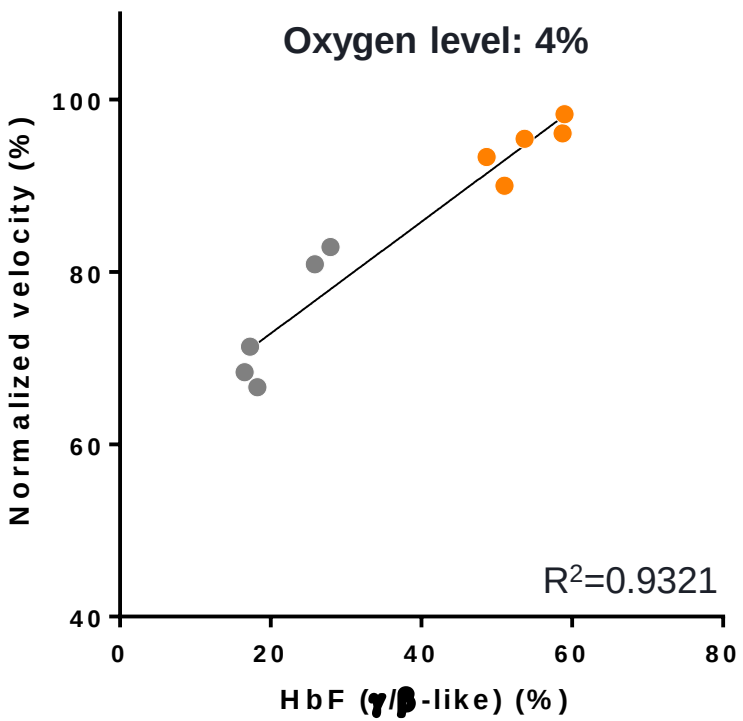
Mean HbF (%): 19.9 53.8

When exposed to sodium metabisulfite

Improved rheological behavior



HbF levels correlated with velocity



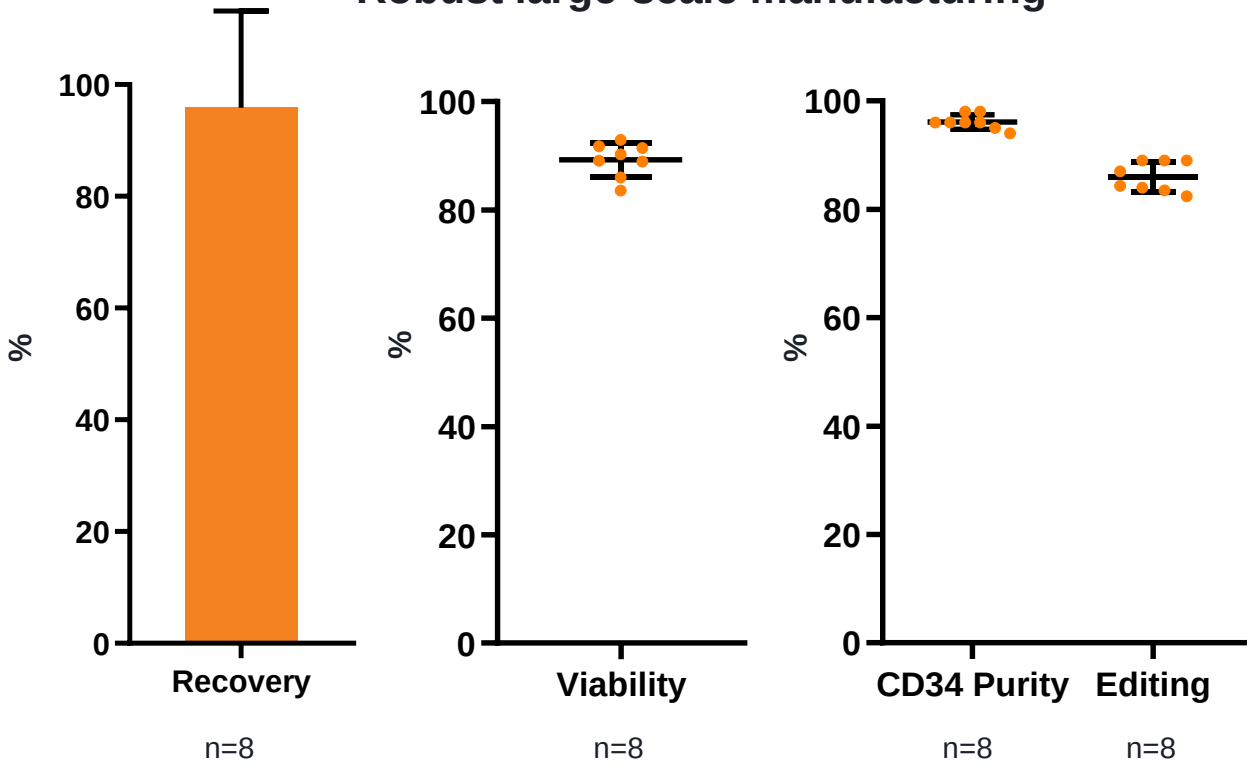
When placed in microfluidic channels, mimicking blood flow in microvasculature, at a range of oxygen levels

◆ Normal donor-derived RBCs ● Unedited SCD-derived RBCs ● EDIT-301 (edited SCD)-derived RBCs

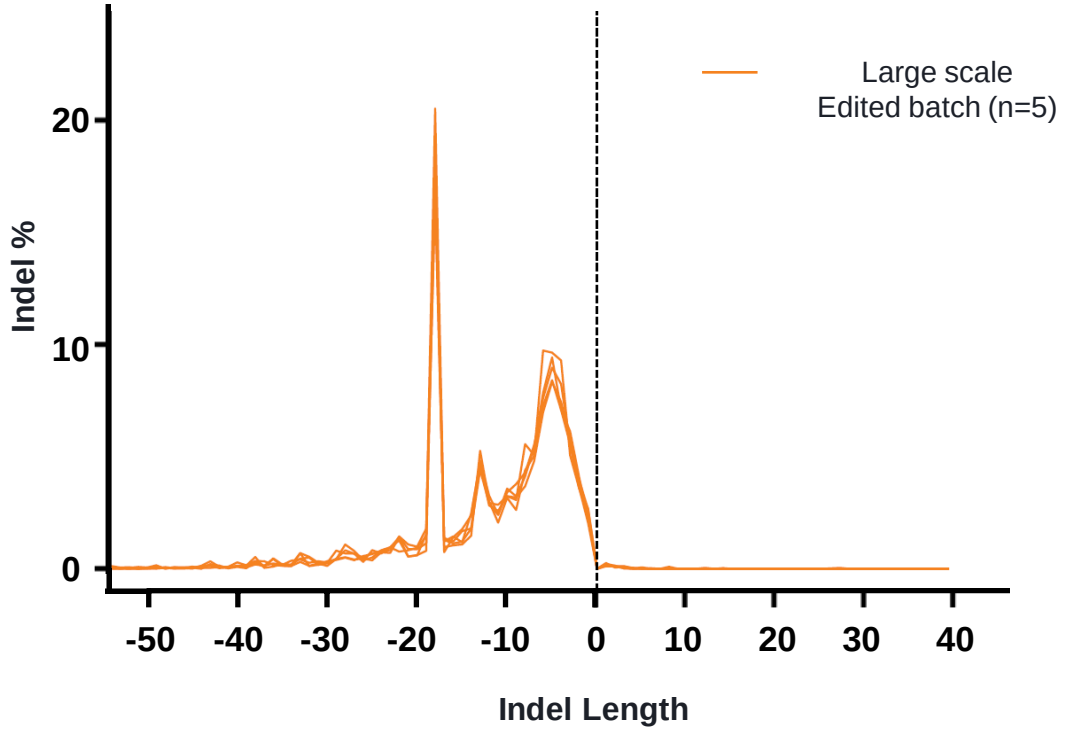
Oxygen (%) 1% = 7.6mmHg

Consistent and robust large-scale manufacturing of edited CD34+ cells from normal donors

Robust large-scale manufacturing

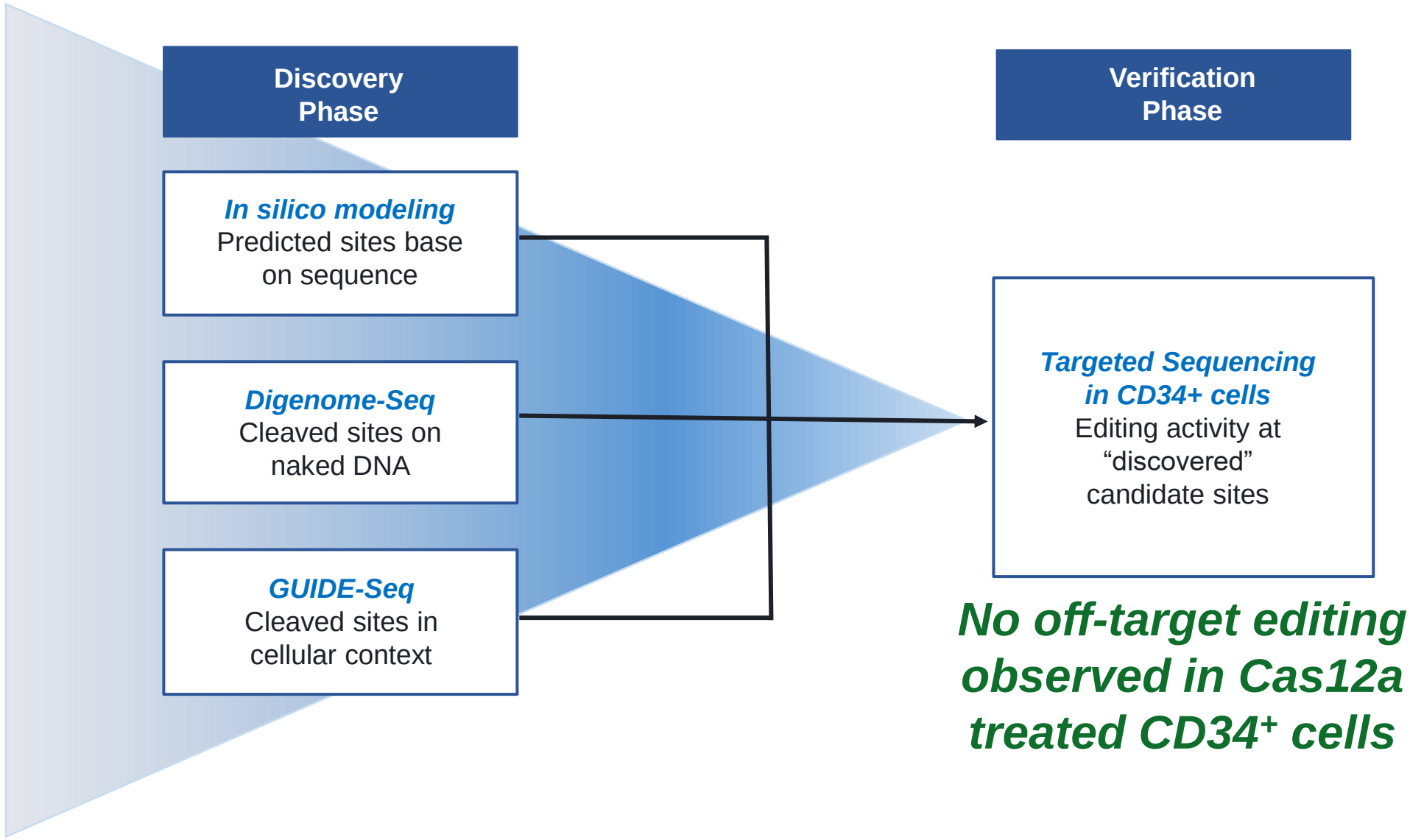


Consistent editing profile



Deletions are represented as negative values ;
Insertions as positive values

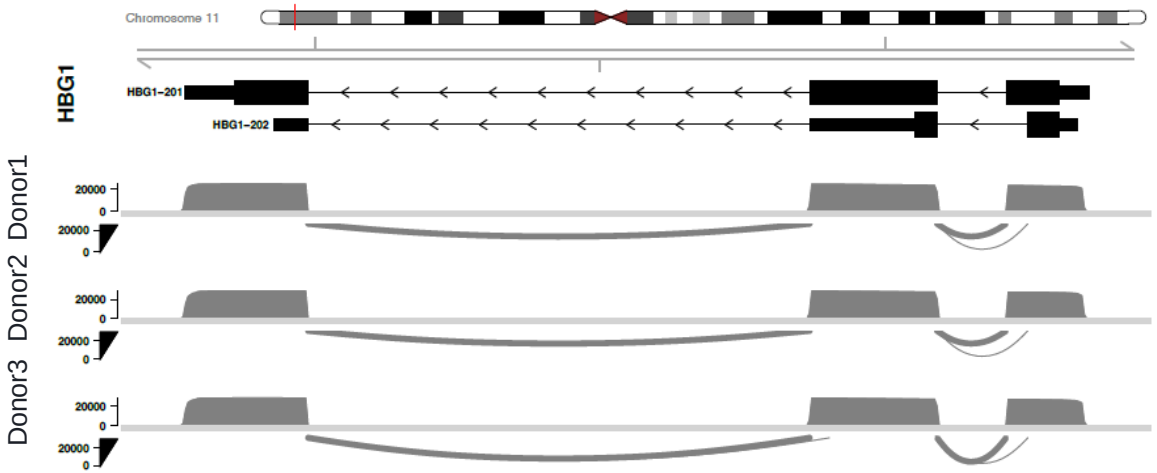
Cas12a RNP is highly specific and no off-target editing was detected in large scale manufacturing batches



No off-target editing observed in Cas12a treated CD34⁺ cells

No detectable unintended globin transcript variants in edited CD34-derived erythroid precursors (Large scale batches)

Large-scale manufacturing – Unedited CD34 derived Erythroid Precursor



Unedited Erythroid Precursors

Edited Erythroid Precursors

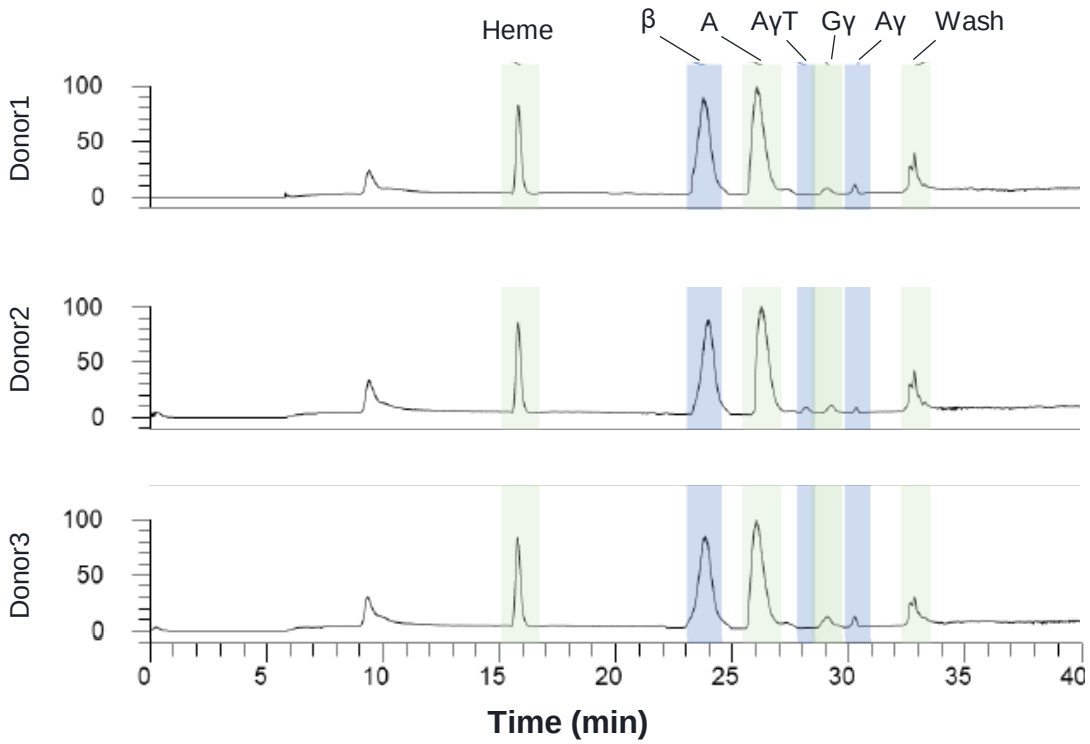
N=3 CD34 normal donors

Large-scale manufacturing – Edited CD34 derived Erythroid Precursors

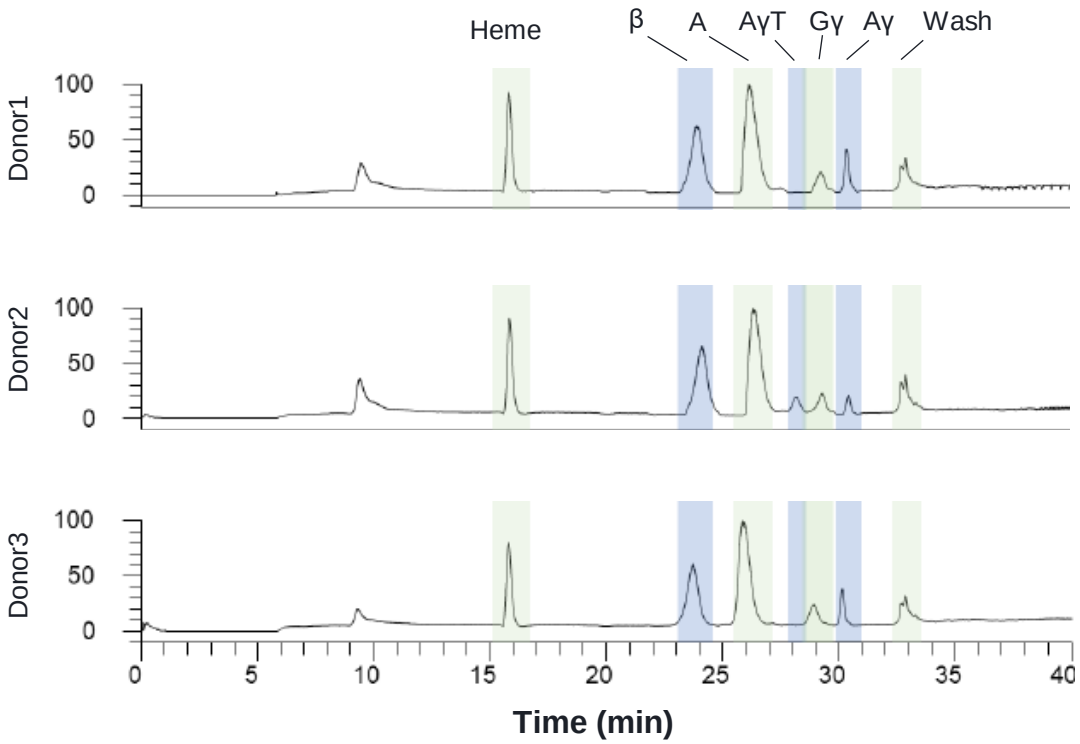
Direct RNA-seq analysis conducted using the Oxford Nanopore System on erythroid cells RNA harvested after 7 days of erythroid differentiation

No detectable unintended globin protein variants in edited CD34-derived RBCs (Large scale batches)

Large-scale manufacturing – Unedited CD34 derived erythroid cells



Large-scale manufacturing – Edited CD34 derived erythroid cells

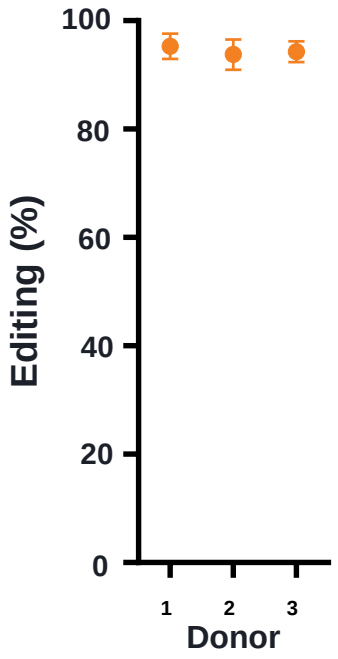


N=3 CD34 normal donors

UHPLC-MS analysis of erythroid cells lysates harvested after 18 days of erythroid differentiation

Infusion of edited CD34+ cells manufactured on a large scale to NSG mice leads to polyclonal engraftment with no lineage skewing

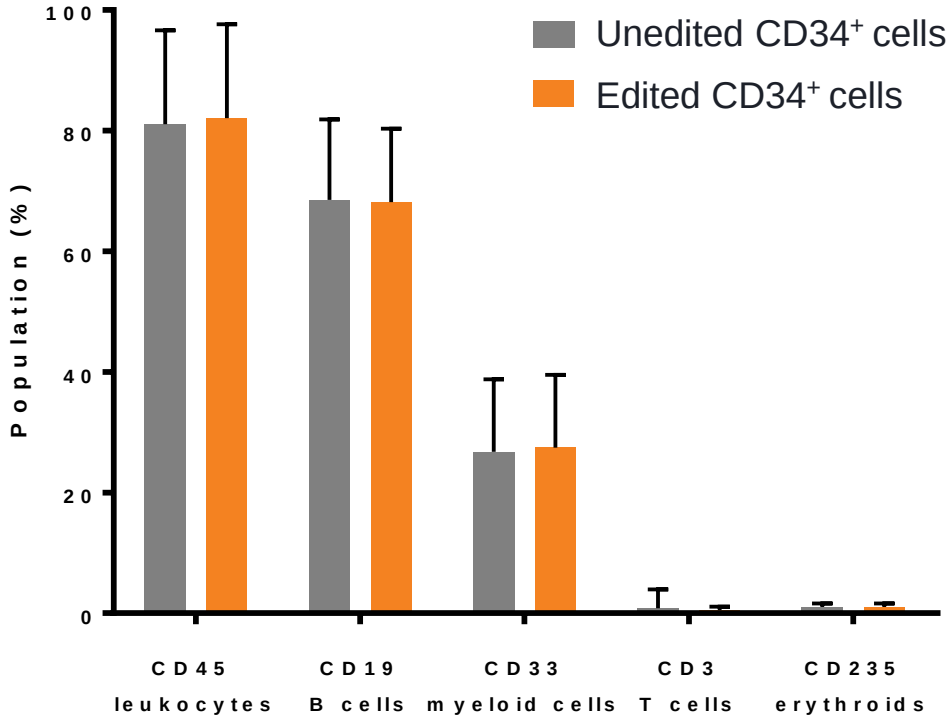
Efficient editing maintained *in vivo*



Bone Marrow at 20 weeks

n=32 mice/donor

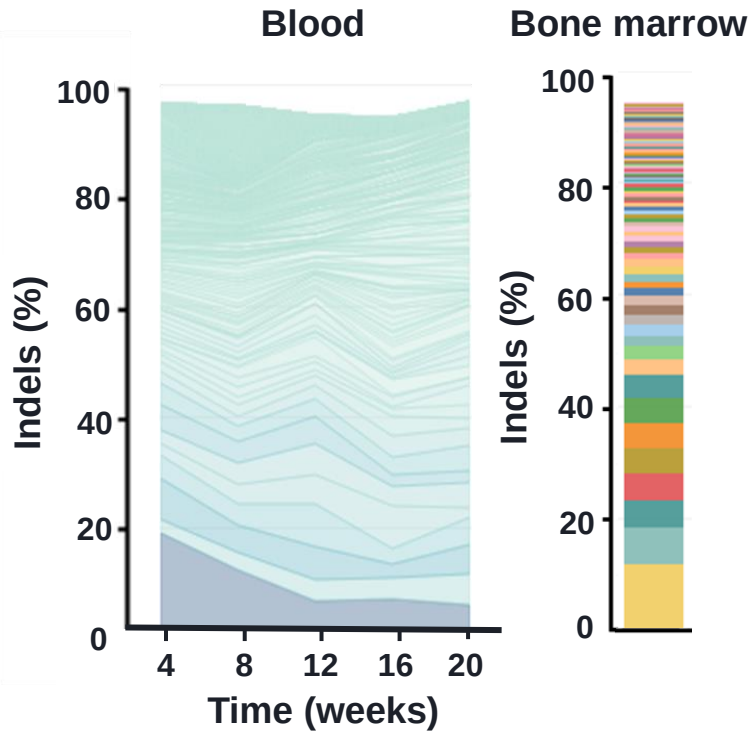
No lineage skewing after engraftment



Bone marrow at 20 weeks post-infusion (Female NSG mice)

n=46–48 mice/treatment (3 donors)

Stable polyclonal engraftment



Blood draws over 20 weeks and BM

Representative data from one NSG mouse. Each color or color shade represents an individual indel signature.

Conclusions

High levels of editing were achieved in CD34^{+} cells using highly specific Cas12a enzyme, leading to **potentially therapeutically relevant levels of HbF** expression

EDIT-301(edited SCD)-derived RBCs demonstrated a **significant reduction in sickling** and **improved rheological properties**

Large-scale process suitable for use in clinical manufacturing led to consistent editing without off-target and unintended HBG variants. Infusion of the edited cells in mice gave rise to multilineage and polyclonal engraftment with persistence of high levels of editing.

These results support the **initiation of the RUBY clinical trial**, a phase 1/2 study of EDIT-301 to treat patients with severe SCD (NCT#04853576).

Developing
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